CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

CHLORHEXIDINE DIACETATE

Chemical Code # 0001144, Tolerance # 50550
SB 950 # 481
Original date: 6/14/01, revised January 15, 2003

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no study submitted
Subchronic, rat, dermal	No data gap, dermal effects at all doses
Chronic toxicity, dog:	Data gap, no study submitted
Oncogenicity, rat:	Data gap, no study submitted
Oncogenicity, mouse:	Data gap, no study submitted
Reproduction, rat:	Data gap, no study submitted
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	Data gap, no study submitted
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	Data gap, inadequate study, no adverse effect indicated
Neurotoxicity:	Not required at this time.

Toxicology one-liners are attached.
All record numbers through 202113 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T030115
Original: J. Kishiyama and J. Gee, 6/14/01, revised by Gee, 1/15/03
See also the "Reregistration Eligibility Decision (RED): Chlorhexidine diacetate", US EPA, September 1996. In that document, US EPA determined that no further toxicity studies were required at that time.
Chlorhexidine is registered as a disinfectant. It also has uses as a preservative in cosmetics and as a surgical scrub. It is irritating to the eye.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

50550 012 202112 "Final report on the safety assessment of chlorhexidine/chlorhexidine diacetate/chlorhexidine dihydrochloride/chlorhexidine digluconate." (Willis, L., publ. in *J. American College of Toxicology* 12: 201 - 223 (1993). This publication was submitted in response to the reviews of studies on file conducted in June of 2001. The review covers a number of areas including uses, animal toxicity, ocular irritation, ototoxicity, dermal irritation and sensitization, mutagenicity, carcinogenicity and clinical assessment. Some of the citations included on toxicity are not on file with the Department. No worksheet. Supplemental information. (Gee, 1/13/03)

CHRONIC TOXICITY, RAT

No study submitted

Subchronic:

** 004, 013 131737, 202113 Henwood, S. M. "13-Week Dermal Toxicity Study with Chlorhexidine Acetate in Rabbits." (Hazleton Laboratories America, Inc., HLA 6247-102, November 28, 1988.) Chlorhexidine acetate, lot R4796, purity not stated, was applied at doses of 0, 250, 500, or 1000 mg/kg/day to the intact dorsal dermal surface of 10 New Zealand White rabbits/sex/group. The test material was applied moistened with 2 ml of water and covered with a gauze dressing and Saran Wrap. Treatment period was 6 hours/day, 5 days/week for 13 consecutive weeks. There were no effects on ophthalmology or hematology. Body weight and food consumption was slightly lower in the first 1 –2 weeks of the study. Aspartate aminotransferase and alanine aminotransferase were increased in females at 500 and 1000 mg/kg, week 14. The incidence of hepatocellular degeneration/necrosis in males was 0, 0, 0, 1 and 0, 1, 5, and 7 in females with increasing dose. Systemic NOEL = 500 mg/kg for males and was not established for females**.** A dermal NOEL was not established as there were dermal effects at all doses in both sexes. Evaluated as unacceptable (rationale for dose selection, particularly for low dose females; test article stability, purity, and characteristics were not reported.). Possibly upgradeable. (Kishiyama and Gee, 6/7/01) Record 202113 contained responses to the deficiencies noted. Test article was 97.7% purity from study records. Concentration analysis was not performed as the test article was formed by adding water to the powder and making a paste. The dose selection was justified as being a limit dose of 1000 mg/kg/day and choosing logarithmically spaced doses for the mid- and low-dermal doses. The age of the animals was approximately 12 weeks from the date of birth on the invoice. The liver necrosis was graded as minimal in all animals and the incidence of 1/10 at 250 mg/kg was not statistically significant by Fishers exact test. The study is now considered ACCEPTABLE. (Gee, 1/14/03)
EPA considered the systemic NOEL to be 250 mg/kg/day based on liver findings at 500 mg/kg in females (See RED). The study was rated as "core-minimum" in 1989.

CHRONIC TOXICITY, DOG

No study submitted

ONCOGENICITY, RAT

No study submitted

ONCOGENICITY, MOUSE

No study submitted

REPRODUCTION, RAT

No study submitted

TERATOLOGY, RAT

** 008, 013 131738, 202113 Lamb, I. C. "A Developmental Toxicity Study of Chlorhexidine Diacetate in Rats." (WIL Research Laboratories, Inc., WIL-173001, October 10, 1991). Chlorhexidine diacetate, purity 98.32%, was administered via gavage at doses of 0 (distilled water), 15.63, 31.25, and 62.50 mg/kg/day to 25-mated Charles River Crl: CD®BR female rats once daily from gestation day 6 through 15. There were minor effects on body weight gain and food consumption in the treated groups compared with controls. Clinical signs of rales and salivation were observed in all chlorhexidine diacetate treatment groups with an apparent increase in incidence with increasing dose (incidences of rales were 0, 4, 4, and 6 with increasing dose; salivation incidences were 0, 1, 4 and 8/25). These signs were seen with greater frequency during the dosing period when observations were made one hour after dosing. In several instances, the signs were associated with expulsion of test material. The signs may have been due to local effects rather than systemic toxicity. Maternal NOEL < 15.63 mg/kg/day (clinical signs). Developmental NOEL = >62.50 mg/kg/day. Evaluated as unacceptable, possibly upgradeable (dose selection justification). (Kishiyama and Gee, 6/15/01). Record 202113 summarizes the bases for the selection of the high dose at 62.5 mg/kg/day based on earlier studies. It did not, however, address the apparent lack of a maternal NOEL. There was a suggestion that the maternal clinical signs may have been a local effect rather than systemic. The study is considered adequate for testing for developmental effects. ACCEPTABLE. (Gee, 1/14/03)

005 131739 This document is the review of the above study by US EPA, dated 1991. The evaluation concluded that the study was "CORE-Minimum" with a developmental NOEL of 62.5 mg/kg (HDT) and a maternal NOEL of 15.63 mg/kg, based on reduced weight gain and rales. NOTE: The review cites previous submissions for developmental studies in the rat which had been judged as CORE-supplementary (page 4), identified as HED Doc. Nos 007012, 007120 and 008073. These studies are not on file with the DPR.. No worksheet. (Gee, 6/7/01).

TERATOLOGY, RABBIT

No study submitted

GENE MUTATION

002 038099 Kuzolas, C. G. "Mutagenicity Test". (Raltech Scientific, RT No. 728366, July 5, 1979.) Chlorhexidine Diacetate, lot R-64, was tested at concentrations of 0, 5, 10, 50, 500, or 1,000 ug/plate with and without metabolic activation (S9 Mix) for mutagenicity using *Salmonella typhimurium* strains TA 98, TA100, TA1535, TA1537 and TA1538. The assay was also performed as a "spot test". Study was UNACCEPTABLE (insufficient information). Not upgradeable. Most concentrations used were too toxic for data collection. (Kishiyama and Gee, 6/4/01).

002 038100 "Mutagenicity Test". (Raltech Scientific, RT No. 748579. September 5, 1980.) Chlorhexidine diacetate, lot R-64, was tested at concentrations of 0, 2, 3, 4, 5, or 6 µg/plate with and without metabolic activation (S9 Mix) for mutagenic potential using *Salmonella typhimurium* strains TA 98, TA100, TA1535, TA1537 and TA1538. No increase in the number of revertants was reported with Chlorhexidine Diacetate. Study was UNACCEPTABLE (Insufficient information - Summary report, data only). Not upgradeable. (Kishiyama and Gee, 6/4/01).

003 045639 Draus, M. "Mouse Lymphoma Forward Mutation Assay: Chlorhexidine Hydrochloride". (Hazleton Laboratories America, Inc., Project No. 2191-100, November 1, 1982.) Chlorhexidine hydrochloride [not diacetate], lot S-80, at concentrations of 0, 0.4, 0.9, 2, 5, 16, 32, 63, and 90 µg/ml with and without metabolic activation (S9 Mix) was evaluated for the potential to induce forward mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. There was a single culture per incubation condition with triplicate plates for mutation frequency and for plating efficiency in a single trial. Chlorhexidine hydrochloride at 0.9 and 2 µg/ml without S9 Mix and at 16 µg/ml with S9 Mix increased forward mutations at the TK locus in L5178Y cells. UNACCEPTABLE. Not upgradeable (single trial). Also, the test article was not characterized). **Possible adverse effect**. (Kishiyama and Gee, 6/4/01).

** 003, 013 045642, 202113 Cifone, M. A., Study Director. " Mutagenicity Evaluation of Chlorhexidine Hydrochloride in the Mouse Lymphoma Forward Mutation Assay." (Litton Bionetics, Inc., LBI Project No.: 20989, October, 1984.) Chlorhexidine Hydrochloride [not diacetate], purity 99.23%, was evaluated for mutagenic potential at concentrations of 0 (DMSO) and ranging from 0.25 to 16.0 µg/ml for 4 hours exposure time, with and without metabolic activation, using mouse lymphoma L5178Y cells. Concentrations were selected based on solubility and cytotoxicity. There were duplicate cultures per concentration with two trials. Each culture, following a 2-day expression period, had three dishes for mutation frequency and three for cloning efficiency. Positive controls were ethylmethane sulfonate without activation and 3-methylcholanthrene with activation, both at two concentrations and both functional. There was no significant increase in the mutation frequency with chlorhexidine hydrochloride treatments in this study. Evaluated as unacceptable (no justification for using the hydrochloride). Possibly upgradeable. (Kishiyama and Gee, 6/6/01). Record 202113 contains a statement regarding the use of the hydrochloride salt in the study. The chlorhexidine moiety is the active portion and the salts differ in solubility. The study is upgraded to ACCEPTABLE status with no adverse effect identified. No supplemental worksheet. (Gee, 1/13/03)

Note: There were conflicting results with mouse lymphoma cells assayed with chlorhexidine hydrochloride at two different laboratories. Based on the quality of the studies, the weight of evidence has been given to the study conducted at LBI. (Gee, 1/13/03).

CHROMOSOME EFFECTS

** 003, 013 045641, 202113 Farrow, M. G., Study Director. "*In Vitro* Chromosome Aberrations in Chinese Hamster Ovary Cells with Chlorhexidine Hydrochloride." (Hazleton Laboratories America, Inc., Project No. 2191-101, February 14, 1983.) Chlorhexidine hydrochloride [not diacetate], purity not stated, was evaluated for mutagenicity at concentrations of 0 (DMSO), 0.1, 0.3, 1.0, 3.3, and 10 μg/ml for 2 hours exposure time with metabolic activation and for ten hours without activation using Chinese hamster ovary cells. There were duplicate flasks per concentration with two slides prepared from each flask. Approximately 100 metaphases were scored per concentration. The positive controls were mitomycin C without activation and cyclophosphamide with activation. Both were functional. No increase in the frequency of chromosomal aberrations was observed at any of the concentrations tested with or without metabolic activation. Evaluated as unacceptable. Possibly upgradeable (test article stability and purity, justification for use of the hydrochloride, individual data and information of the activation source). (Kishiyama and Gee, 6/6/01). Record 202113 addressed the deficiencies noted in the initial review. The active portion of the test article is the chlorhexidine and the salts determine solubility. The purity was no less than 95.6%. Since the positive control, cyclophosphamide, was active, the S9 activation system was functional, despite the lack of details. Also, in the preliminary trial, there was a 50% reduction in cell growth at 10 μg/ml, so the test system responded to the test article. Although these responses are less than initially requested, there is no need to repeat the study, which is upgraded to ACCEPTABLE status with some deficiencies. (Gee, 1/14/03)

DNA DAMAGE

003, 013 045640, 202113 Myhr, B. C. , Study Director. "Evaluation of Chlorhexidine Hydrochloride in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay." (Litton Bionetics, Inc., LBI Project No. 20991, January, 1983.) Chlorhexidine Hydrochloride [not diacetate], purity not stated, was evaluated for mutagenicity at concentrations of 0, 0.0242, 0.0484, 0.0968, 0.242, 0.484, 0.968, 2.42, and 4.84 μg/ml for 18 – 19 hours using rat hepatocytes, triplicate coverslips per concentration. No significant change in the nuclear labeling of rat hepatocytes was reported. Chlorhexidine was cytotoxic at 2.42 ug/ml and above. No adverse effect. Evaluated as unacceptable (test article purity and stability not reported, no individual data, and no justification for using the hydrochloride). Possibly upgradeable. (Kishiyama and Gee, 6/4/01). Record 202113 contains the information that the lot was S-140 with a minimum purity of 95.6%. The chlorhexidine moiety is the active portion and the salts only affect the solubility. No additional data regarding the results were included in the record but it contains a statement that photocopies could be supplied. The study remains UNACCEPTABLE but upgradeable with submission of the individual cell data. (Gee, 1/13/03)

ACUTE STUDIES

** 006 131740 Reagan, E. L. "Acute Oral Toxicity Study of Chlorhexidine Acetate in Sprague-Dawley Rats." (Food and Drug Research Laboratories, FDRL Study No.: 89.1810.003, October 23, 1989.) Chlorhexidine Acetate, 99.5%, Lot 3-P, was administered as a single dose by gavage at 2000, 2646 or 3500 mg/kg to 5 Sprague-Dawley rats/sex/group. Animals were observed for 15 days for treatment-related effects and mortality. Clinical observation and necropsy indicate treatment-related effects at all doses. No NOEL. Calculations from cumulative mortality data showed the combined LD_{50} = 2646 mg/kg (males =

2292 [1403 – 3180 mg/kg] and females = 3055 mg/kg [1870 – 4239 mg/kg]). Category III. ACCEPTABLE. (Kishiyama and Gee, 6/12/01).

** 006 131748 Reagan, E. L. "Acute Dermal Toxicity Study of Chlorhexidine Acetate in New Zealand White Rabbits**".** (Food and Drug Research Laboratories, FDRL Study No.: 89.1810.004, August 2, 1989.) Chlorhexidine Acetate, purity 99.50%, was applied topically for a period of 24 hours at 2000 mg/kg to 5 New Zealand White rabbits/sex. The material was moistened with saline, covered with gauze and occluded. Animals were observed for a period of 15 days. Clinical observation indicated treatment-related effects of dry skin at the application site. Test article caused dermal irritation (erythema, edema and dry skin). All animals survived to terminal sacrifice. Dermal LD_{50} > 2000-mg/kg. Category III. ACCEPTABLE (Kishiyama and Gee, 6/12/01)

** **006 131749** Reagan, E. L. "Primary Eye Irritation Study of Chlorhexidine Acetate in New Zealand White Rabbits". (Food and Drug Research Laboratories, FDRL Study No. 89.1810.005, August 2, 1989.) One-tenth gram of chlorhexidine acetate, purity 99.50%, was placed in the conjunctival sac of one eye of each of 6 New Zealand White rabbits. Eyes were examined at 1, 24, 48 and 72 hours and at 4 and 7 days after dose administration. **Test animals suffered severe ocular irritation with chlorhexidine acetate treatment** , which resulted in their sacrifice for humane reasons on day 7 of the study. Category I. ACCEPTABLE. (Kishiyama and Gee, 6/12/01).

** 006 131750 Reagan, E. L. "Primary Dermal Irritation Study of Chlorhexidine Acetate in New Zealand White"**.** (Food and Drug Research Laboratories, FDRL Study No.: 89.1810.006, August 2, 1989.) One-half gram (0.5 g) of chlorhexidine acetate, purity 99.5%, moistened with 0.5-ml physiological saline, was applied topically to the shaved area of 6 New Zealand White rabbits, 3/sex, for 4 hours. A gauze was applied after dosing to protect the treated area for the 4 hours, after which the gauze removed and the treated area cleaned. Dermal irritation was evaluated at 0.5, 24, 48 and 72 hours after removal of the protective gauze. No dermal irritation was reported up to 72 hours following test article treatment for any of the test animals. Dermal Toxicity Category IV. ACCEPTABLE. (Kishiyama and Gee, 6/13/01).

** 006 131751 Biesemeier, J. A. "Dermal Sensitization Study of Chlorhexidine Acetate in Guinea Pigs." (Food and Drug Research Laboratories, FDRL Study No.: 89.1810.007, August 22, 1989.) One-half gram [0.5 g] of chlorhexidine acetate, purity 99.5%, lot 3-P, moistened with 0.5 ml of 80% ethanol, was applied topically three times a week for 3 weeks to the shaved area of 5 albino guinea pigs/sex for 6 hours, using a modified Buehler method. The treated area was covered with a gauze, which was removed after the 6 hours, and the treated area cleaned. After a two week rest period, the test animals were re-treated (24 hour exposure) on a virgin area of the back, along with 4 (2/sex) naive animals, to a challenge dose of 0.5 g. The scores of erythema was from no reaction to strong erythema (1 male, day 7) with chlorhexidine acetate and from slight to strong erythema with DNBC treatments. The results from the challenge application indicated no meaningful difference between chlorhexidine acetate and naive control. Incidence scores were 0 for both groups and the severity index was 0.1 for the treated group and 0.3 for naïve controls at 26 hours and 0.1 for treated groups and 0 for controls at 48 hours. DNBC functioned with severity indices of 1.6 and 0.8 at 26 and 48 hours. Chlorhexidine acetate was not a sensitizer. ACCEPTABLE . (Kishiyama and Gee, 6/13/01).

** **007 131752** Shapiro, R. "EPA Acute Inhalation Toxicity - Defined LC_{50}". (Product Safety Labs, Lab. Proj. I.D. T-1813, January 28, 1993.) Chlorhexidine Diacetate, purity 97.5 to 101%, was administered as an aerosol at concentrations of 0.10, 0.46 and 5.09 mg/L to 5 rats/sex/group for 4 ½ hours for the low and mid concentrations. Complete mortality occurred at the high dose within 2 hours. MMAD (GSD) were: 2.1 (2.15), 2.4 (2.19), and 2.6 (2.16) µm for low through high concentrations. Mortality was 100% (within 2 hours), 90% and 0% for high, mid, and low-doses, respectively. All surviving animals were observed for 14 days and recovered from the treatment-related clinical effects. All test animals had discolored lungs and in addition, for those that died, tracheas filled with mucous, corneal opacity, discolored and gaseous distention of the G.I. tract. LC_{50} was 0.30 mg/L (0.12 to 0.77 mg/L) and 0.43 mg/L (0.18 to 1.07 mg/L) for males and females, respectively. Inhalation Toxicity **Category II**. ACCEPTABLE. (Kishiyama and Gee, 6/14/01)